IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Michael SCHIRNER et al.

Serial No.:	10/088,866	Examiner:	HUFF, Sheela Jitendra
Filed:	July 2, 2002	Group Art Unit:	1642

Title: ANTIBODY-DYE CONJUGATES FOR BINDING TO TARGET STRUCTURES OF ANGIOGENESIS IN ORDER TO INTRAOPERATIVELY DEPICT TUMOR PERIPHERIES

DECLARATION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Kai Licha, being duly warned, declare that:

I am a citizen of Germany.

I am a co-inventor of the present application and also of the reference US 6,083,485, to Licha et al., used in obviousness rejections.

If a patent issues from this application, and the assignee decides to pursue a commercial product falling under said patent's claims, and said commercial product is approved by FDA and sold in the U.S., then under German law, I and the other inventors will receive some income derived from such sales.

I am familiar with the obviousness rejections in the Office Action dated October 18, 2006, and the references cited therein.

I conducted or supervised the experiments discussed below:

COMPARATIVE ANALYSIS OF ANTIBODY-DYE-CONJUGATES

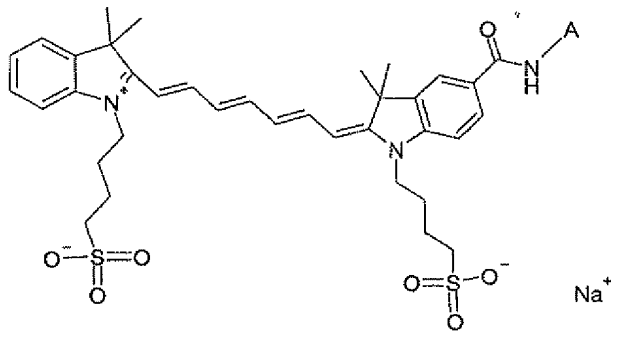
These experiments compare antibody-dye conjugates according to the invention with the scFv-Cy7 conjugate of Neri et al., Nature Biotechnology Vol 15 p. 1271 (11/97). The antibody portion is an scFv in each of the conjugates.

1) Labelling of Anti-ED-B-Fibronectin scFv Antibodies (A) with dyes 1-4 to form conjugates 1-4, respectively, of the claimed invention, and with conventional dye Cy7-bisfunctional NHS ester (Amersham Pharmacia Biotech) of Neri et al.

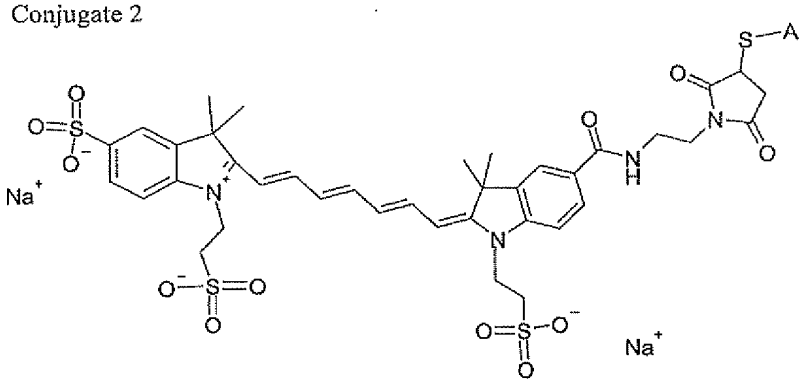
0.3 ml of a solution of the antibody in PBS (conc. 1.2 mg/ml) was mixed with the dye (start solution: 0.5 mg/ml in PBS) and incubated for 4 h at 25°C. The conjugate was separated by gel filtration on NAP-5 column (eluant: PBS/10% glycerine).

The following conjugates were produced:

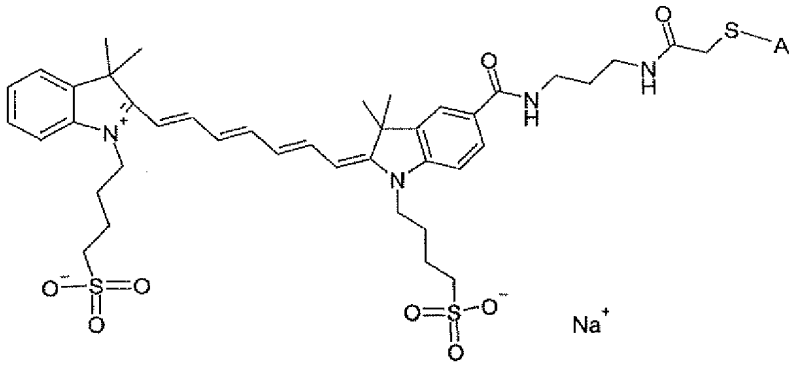
Conjugate 1



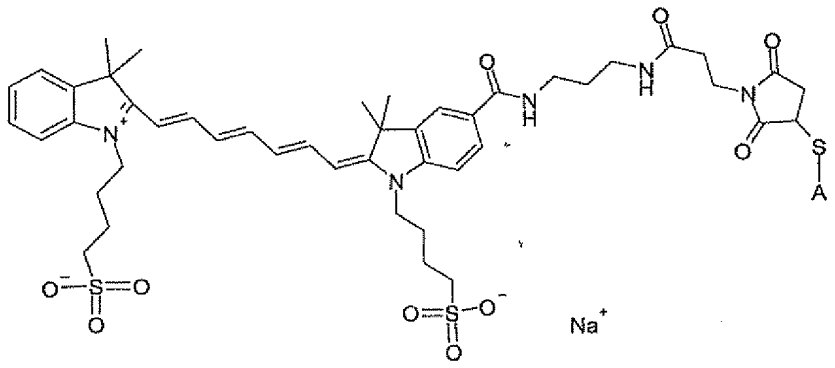
Conjugate 2



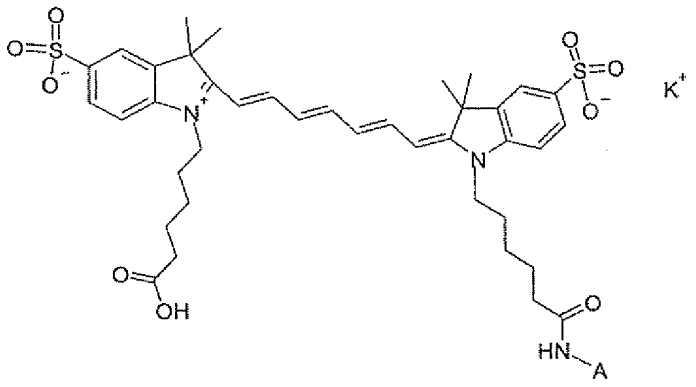
Conjugate 3



Conjugate 4



Conjugate 5 (Conjugate of Neri et al.)



2) Determination of the immune reactivity

The immune reactivity of the conjugate solution was determined by affinity chromatography (ED-B-fibronectin resin) (*J Immunol Meth* 1999, 231, 239).

3) Photophysical Characterization of the Dye-Antibody-Conjugates

The load factor (dye/antibody molar ratio) was determined by photometry and based on an extinction coefficient of 75000L mol^{-1} in the short-wave absorption range (about 690 nm); the antibody absorption was determined with an OD_{280nm} of 1.4. The fluorescence quantum yield was determined with SPEX fluorolog (wavelength-dependent sensitivity calibrated by lamp and detector) relative to Green Indocyanine (Q=0.13 in DMSO, *J Chem Eng Data* 1977,22,379; *Bioconjugate Chem* 2001,12,44).

The results are provided in the table below:

Substance	Load factor	Absorption Maximum (nm)	Immuno-reactivity %	Fluororescence Quantum Yield
Conjugate 1	1.0	750	65	0.15
Conjugate 2	1.1	752	90	0.075

Conjugate 3	1.5	750	77	Not Measured
Conjugate 4	1.2	749	75	0.11
Conjugate 5 (of Neri et al.)	2.3	751	11	0.027

The data demonstrate that for all 4 of the tested conjugates of the present invention, unexpectedly, and significantly improved immunoreactivity is achieved over the conjugate of Neri et al., and that for 3 of the claimed conjugates, unexpectedly, and significantly increased fluorescence quantum yield is achieved in comparison to the conjugate of Neri et al.

The data demonstrate that the conjugates of the invention provide a significantly better immunoreactivity, which in practice leads to better tumor imaging efficacy, over the comjugate of Neri et al. Such a result was not expected from the disclosure of the prior art. Accordingly, the claimed invention is not obvious.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

17.07.2007

Date

Dr. Kai Licha